Supplemental materials

C57BL/6J (wild-type) mice (CLEA Japan, Inc., Tokyo, Japan) were housed in standard plastic cages at an animal facility maintained at 23°C–25°C and 50%–56% humidity under a 12/12 h light/ dark cycle (lights on 8:00–20:00). The mice had free access to tap water and standard laboratory rodent food (MF diet, Oriental Yeast Co., Ltd., Tokyo, Japan). The animal experiments adhered to the guidelines for maintenance and handling of experimental animals established by the Tokyo University of Agriculture Ethics Committee.

Jejuna were collected from the mice euthanized by cervical dislocation. The luminal contents were thoroughly washed with ice-cold phosphate-buffered saline (PBS). The jejuna were longitudinally opened and placed on a cold stainless-steel tray with the luminal side facing upward. Each jejunum (approximately 1 cm) was embedded with O.C.T. compound (Sakura Finetek Japan Co. Ltd., Tokyo Japan) at −80°C before cutting into 12-μm sections with a cryostat (Leica CM3050S, Leica Biosystems, Wetzlar, Germany). The frozen sections were fixed on MAS-coated glass slides (Matsunami Glass Ind., Ltd., Osaka, Japan). Subsequently, immunohistochemistry was performed using anti-Reg3β primary antibody (Cat. No. PA5-47700, Thermo Fisher Scientific Inc., MA, USA) and donkey anti-sheep IgG (H+L) crossadsorbed secondary antibody conjugated with Alexa Fluor® 488, (Cat No. A-11015, Thermo Fisher Scientific Inc.).

For *in situ* hybridization, a mouse Reg3β cDNA fragment (457 bp) was cloned by RT-PCR using cDNA from the small intestine and specific primers for Reg3β (Forward: TGCCTCCACAGCCTGCTC, Reverse: CCAGAACGTCTTGACAAGCTGC). Adult mouse jejuna frozen in the O.C.T compound were cut into 12-μm thick sections and subjected to postfixation (10 min in

4% paraformaldehyde, followed by acetylation in acetic anhydride for 10 min). After three washes in PBS, the sections were prehybridized in a hybridization buffer (5x standard saline citrate, 50% formamide, 1x Denhardt's solution, 1 mg/mL salmon sperm DNA, 1 mg/mL tRNA). Hybridizations were performed with digoxigenin-labeled cRNA probes in the hybridization buffer for 18 h at 70°C. Hybridization signals were detected with alkaline phosphatase-conjugated anti-digoxigenin antibodies plus NBT/BCIP substrate (Merck & Co., NJ, USA).

Enteroids were isolated from fresh jejunal crypts obtained from the mice as described previously [13]. Briefly, the animals euthanized by cervical dislocation were dissected, and the jejuna were collected. The luminal contents were thoroughly washed with ice-cold PBS. The jejuna were longitudinally opened and placed on a cold stainless-steel tray with the luminal side facing upward. The villi were scraped with a scalpel before the jejuna were agitated in 10 mL of 30 mM ethylenediaminetetraacetic acid-3Na/Hanks' balanced salt solution (HBSS) for 10 min at room temperature. Each jejunum was then placed into a tube comprising 10 mL of HBSS. The tube was shaken 20 times, and the first fraction was subsequently collected. Then, the jejunum was transferred to a separate tube comprising 10 mL of ice-cold HBSS. The tube was shaken 50 times, and the second fraction was subsequently collected. Similarly, the third and the fourth fractions were collected by transferring the jejunum to tubes comprising 10 mL of ice-cold HBSS and shaking the tube 50 and 100 times, respectively. Each fraction was centrifuged at 400 x *g* at 4°C for 4 min, and the supernatant was subsequently removed. Pellets from each fraction were individually suspended in 500 μL of 10 mM Y-27632/HBSS. After counting the crypts and villi using a microscope, crypt purity was calculated using the following formula: crypt purity (%) = crypts/(villi + crypts). Samples with purity of >80% were centrifuged at 400 x *g* at 4°C for 4 min.

The supernatants were removed, and the crypts (200 crypts/well) were embedded in 60% Matrigel® (BD Biosciences, San Jose, CA) (40 μL/well) in a 24-well plate (Nippon Gene Co., Ltd., Tokyo, Japan). The Matrigel® suspension was allowed to polymerize at 37°C for 10 min before adding fresh Advanced DMEM/F12 comprising 1 mM L-alanyl-L-glutamine, B-27 (Thermo Fisher Scientific Inc., MA, USA), 1 mM N-acetyl-L-cysteine, penicillin/streptomycin, 10 mM HEPES (NACALAI TESQUE, Inc., Kyoto, Japan), 10% R-spondin conditioned medium (CM), 5% Noggin CM, and 50 ng/mL recombinant murine EGF (Funakoshi Co., Ltd., Tokyo, Japan). This medium was defined as basic medium, which was replaced every 2–3 days. Enteroids from the mice were collected using ice-cold PBS, mechanically disrupted by passage through a syringe with a needle (27G) (Terumo Corporation, Tokyo, Japan), and subsequently transferred to fresh 60% Matrigel®. The enteroids were passaged every 4–6 days.

For the mRNA expression experiments, on day 3 of culture, the enteroids were stimulated with IL-22 (0, 0.01, 0.1, 1, and 10 ng/mL) at final concentrations for 1, 3, 9, and 24 h. Total RNA was isolated from the enteroids using ISOGEN II (Nippon Gene Co., Ltd.). cDNA was synthesized using a PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (Takara Bio Inc., Shiga, Japan) according to the manufacturer's instructions. qPCR was conducted in an ABI PRISM 7300 Sequence Detection System (Thermo Fisher Scientific Inc.) using THUNDERBIRD® SYBR® Green and primer sets [14]. The PCR conditions were 30 s at 95°C and 40 cycles of 5 s at 95°C and 30 s at 60°C.

For immunohistochemical staining of enteroids [13], the enteroids were transferred to an 8-well glass bottom chamber (Thermo Fisher Scientific Inc.) on day 2 of culture and cultured in the basic medium comprising IL-22 at a final concentration of 10 ng/mL for 24 h. After removing the medium, 200 μL of 5 μg/mL Hoechst 33342

(Thermo Fisher Scientific Inc.) in PBS was added to each well, and the plates were incubated for 30 min under 5% CO_2 at 37°C. After the Hoechst solution was removed, the enteroids were fixed in 200 μL of 4% paraformaldehyde in PBS for 30 min at room temperature in the dark before washing three times with 200 μL of PBS for 20 min. The enteroids were blocked in 200 μL of a blocking buffer (PBS comprising 2% donkey serum and 0.3% Triton X-100) for 1 h at 4°C in the dark. The enteroids were then incubated with 200 μL of primary antibodies at 4°C overnight in the dark. The antibodies used were anti-Reg3β antibody (Cat. No. PA5-47700, Thermo Fisher Scientific Inc.) and anti-lysozyme antibody (Cat No. A0099, DAKO A/S, Glostrup, Denmark). After three 30-min washes with 200 μL PBS, the enteroids were incubated with 200 μL donkey anti-rabbit IgG (H+L) highly crossadsorbed secondary antibody conjugated with Alexa Fluor 555 (Cat. No. A-31572, Thermo Fisher Scientific Inc.) or donkey anti-sheep IgG (H+L) crossadsorbed secondary antibody conjugated with Alexa Fluor 488 (Cat No. A-11015, Thermo Fisher Scientific Inc.) at 4°C overnight in the dark. The enteroids were then washed twice with 200 μL of PBS and subsequently treated with a SeeDB [15] optical clearing agent. The enteroids were analyzed using a confocal laser scanning microscope (FLUOVIEW FV10i, Olympus Corporation, Tokyo, Japan).

Statistical analyses were conducted using the SPSS 15.0J software. The data were analyzed using Student's t-tests or Tukey’s multiple comparison tests, and the results were expressed as the means ± standard error (SE).